Applicants appreciate the Examiner's comments set forth in paragraph 1 of the Action, and enclose herewith certified copies of Australian applications PP0627 and PP6096.

The rejection under Section 112, first paragraph, is respectfully traversed. Applicants respectfully disagree with the positions set forth at items 2 and 3 of the Action and submit that the specification indeed enables any person skilled in the art to make or use the invention, in a manner commensurate with the scope of the pending claims. The skilled person in the art can make and use the claimed invention by employing the teachings of the application, coupled with additional information well known in the art, and without undue experimentation.

In support, Applicants provide the following comments in relation to:

1. The unpredictability of the art
2. The state of the art
3. Number of working examples
4. Scope of the claims
5. Amount of guidance provided by the applicant
6. Nature of the invention
7. Level of skill in the art

These will be examined in turn.

Unpredictability and State of the Art (1 & 2):

Applicants *do not* agree that the construction of flavivirus replicons is "unpredictable", or that it represents a "poorly developed" state of the art. Although flaviviruses share little sequence homology, all the motifs in RNA and in proteins essential for RNA replication are highly conserved. See, for example, the following three journal articles with respect to the construction of similar replicons from other flaviviruses (copies enclosed):

1. West Nile virus (Yamshchikovv, *et al.*, Virology, 281, 294 (2001))
2. Yellow fever virus (Barba *et al.*, "Bicistronic Flavivirus replicons based on tallow fever 17D," page 33 of the abstract book from the 6th International Symposium on Hepatitis C and Related Viruses, NIH, Bethesda, Maryland, 6-9 June (1999)); and
3. Barba *et al.*, "Engineering yellow fever 17D for heterologous gene expression and vaccination," P6-5, page 149 of the abstract book of the 19th annual meeting of American Society for Virology, Fort Collins, Colorado, July 8-12 (2000)

In these reports, it can be seen that the authors very closely followed the design for construction of replicons as amply described in the present application. This included, for example, retaining at least 60 nucleotides of the core protein coding region. Applicants, however, were the first to establish that this sequence is essential for replication of the replicon RNA (See Khromykh *et al.*, J. Virol. 71, 1497 (1997), previously provided). See also the recent publication Khromykh *et al.*, J. Virol. 75, 6719 (2001), copy enclosed.

In the references cited above it is quite *clearly* demonstrated that an 8-nucleotide sequence included into these 60 nucleotides of core protein coding region represents a cyclization motif which hybridizes to the complementary cyclization motif in the 3' UTR region to form an RNA structure vital for its replication. The authors of the articles on the West Nile and Yellow Fever replicons also retained at least the last 66 nucleotides of E protein, as Applicants did, since it is common knowledge in flavivirus research that this sequence is coding for the last hydrophobic domain of E protein and serves as the signal peptide for proper translocation of the next protein, NS1, through the membrane of the endoplasmic reticulum.

Although the packaging mechanisms for flavivirus RNA were unknown at the time of the invention, the present invention clearly demonstrates that the expression of structural proteins in trans from another expression vector was sufficient to allow packaging of co-transfected flavivirus replicon RNA to occur with relatively high efficiency. Although the examples are directed to the use of Kunjin virus components in this packaging system, the strategy is quite clearly applicable to any other flavivirus. In fact, a later report (Barba *et al.*, reference 3 above) demonstrated the feasibility of this approach by presenting the results of packaging of Yellow Fever replicon by the structural proteins expressed from Sindbis replicon vectors.

In contrast to the Examiner's assertions on page 2 of the Office Action, the present application does not claim the development of stable cell lines expressing structural proteins in trans to allow packaging of replicon RNA. Relevant to this, claim 32 claims the use of plasmid DNA as a second vector for transient, continuous or inducible expression of structural proteins; it does not, however, specifically claim the development of stable cell lines expressing structural proteins.

The present application also does not claim stably transformed cell lines capable of expressing flavivirus proteins and supporting flavivirus replication. Instead, the present application claims, *inter alia*, the use of flavivirus replicons with inserted antibiotic resistance genes as vectors for establishing stable cell lines continuously expressing inserted heterologous genes and/or sequences.

Number of working examples and scope of the claims (3 &4):

Although the present examples are concerned primarily with the Kunjin virus, they clearly support claims, such as those presently pending, that are not similarly focused. There is sufficient information in the specification, including the examples, to permit the person skilled in the art to repeat the invention as claimed.

Amount of guidance provided by Applicant (5):

The examples in the present application with Kunjin replicon vectors and Kunjin replicon packaging system present in the invention provide more than enough guidance for the

person skilled in the art of flaviviruses to generate similar replicon expression vectors, cells stably transformed with replicons and replicon packaging systems for any member of the flaviviridae family. This is clearly illustrated by the three reports mentioned above under the heading "Unpredictability and State of the Art."

Nature of the Invention and Level of Skill in the Art (6 & 7):

Please see the comments under "Unpredictability and State of the Art" above, which are applicable to these issues as well.

The rejection under Section 112, first paragraph, as set forth at paragraph 4 of the Action is respectfully traversed. Applicants reassert the comments made in response to items 2 & 3 above, and submit that the Applicants are indeed in possession of the claimed genus.

Finally, the relevant case law has established that a specification need not describe the exact details for preparing every species within the genus described. Instead the test for determining compliance with the written description requirement is whether the disclosure of an application "reasonably conveys" to the artisan that the inventor had possession at that time of the later claimed subject matter. The present application not only meets, but quite clearly exceeds, this threshold.

In view of the above remarks, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of all rejections is thus respectfully requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amendments to the claims (where insertions are underlined and deletions placed in brackets):

1. (once amended) A gene expression and delivery system comprising:

(a) a replicon of flavivirus origin as a first vector, which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; and

(b) at least a second vector that is capable of expressing flavivirus structural protein(s) and/or any other proteins required for packaging of the replicon into infectious flavivirus-like particles [which vector is engineered to prevent recombination with the replicon when in its presence].

6. (once amended) A gene expression and delivery system comprising:

(a) a self-replicating expression vector of flavivirus origin which includes the nucleotide sequence for the flavivirus 5'UTR, at least a portion of the 5' nucleotide coding region for flavivirus core protein, the nucleotide coding region for flavivirus non-structural proteins, a sufficient amount of the 3'-terminal region of the flavivirus 3'UTR required for self-replication of flavivirus genomic material wherein (i) the vector is adapted to receive at least a nucleotide sequence without disrupting the replication capabilities of the vector, (ii) the nucleotide sequence is inserted into the vector in a manner which deactivates expression of at least a gene that would otherwise code for a flavivirus structural protein and (iii) the inserted nucleotide sequence does not encode [for] the structural protein sequence that it deactivates; and

(b) at least a second vector that is [(i)] capable of expressing the flavivirus structural protein(s) that is not expressed by the self-replicating expression vector described in (a) [and (ii)

engineered to prevent recombination with the self-replicating vector described in (a) when in its presence].

11. (once amended) A gene expression and delivery system according to claim 6 wherein the nucleotide sequence is inserted [within the locality]in place of at least a deleted gene encoding a structural protein.

22. (once amended) A gene expression system according to claim 21 wherein the replicon is derived from the FLSD or FLSDX clones [as herein described].

23. (once amended) A gene expression system according to claim 21 wherein the replicon is selected from one of the following vectors: C20rep; C20DXrep; C20DXrepNeo; C20DX2Arep; C20DX2ArepNeo; C20DX/CAT/2Arep; C20DX/CAT/2ArepNeo; C20DXIRESrep; C20DX/CAT/IRESrep; C20DX/GFP/2Arep; C20DX/GFP/2ArepNeo; C20DX/hcvCORE160/2Arep; C20DX/hcvCORE191/2Arep; C20DX/hcvNS3/2Arep; C20DX/VSV-G/2Arep; C20DX/ß-GAL/2Arep; C20DXUb2A_HDVrep or pKUNRep1[, as described herein].

37. (once amended) A gene expression system according to claim 1 wherein the replicon encodes all flavivirus structural proteins except for flavivirus core protein and the second vector is SFV-C [as herein described].

40. (once amended) A flavivirus replicon selected from the following: C20DX2Arep; C20OX2ArepNeo; C20DX/CAT/2Arep; C20DX/CAT/2ArepNeo; C20DXIRESrep; C20DX/CAT/IRESrep; C20DX/GFP/2Arep; C20DX/GFP/2ArepNeo; C20DX/hcvCORE160/2Arep; C20DX/hcvCORE191/2Arep; C20DX/hcvNS3/2Arep; C20DX/VSV-G/2Arep; C20DX/ß-GAL/2Arep; C20DXUb2A_HDVrep or pKUNRep1[; as herein described].

43. (once amended) A method for producing a stable cell line capable of persistently producing replicon RNA'S, comprising the steps of:

(i) introducing into a cell a replicon of flavivirus origin which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region [and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle]; and

(ii) culturing that cell line under conditions which permit cell growth and replication.

44. (once amended) A method for producing a flavivirus like particles comprising the steps of:

(i) introducing into a cell a replicon of flavivirus origin which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region [and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle];

(ii) introducing into a replicon-containing [call]cell a second vector that is capable of expressing flavivirus structural protein(s) and/or any[/or] other proteins required for packaging of the self-replicating expression vector into flavivirus viral particles which vector is engineered to prevent recombination with the self-replicating vector when in its presence; and

(iii) harvesting virus like particles containing the replicon.

46. (once amended) A flavivirus like particle according to claim 45 wherein said particle contains a replicon that is derived from a DNA based replicon vector.

48. (once amended) A DNA based replicon vector of flavivirus origin, wherein the vector comprises: (a) a complementary DNA sequence of flavivirus origin that is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is

unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; (b) a mammalian expression promoter[;] 5' to the complementary DNA sequence of flavivirus origin; and (c) at least a second nucleotide sequence capable of terminating transcription of replicon RNA with a precise 3' terminus; and wherein the promoter and the second nucleotide sequence are capable of promoting transcription and terminating same, of flavivirus RNA within the nucleus of a transfected cell.

49. (once amended) A DNA based replicon vector according to claim 48 wherein the complementary DNA sequence of the nucleotide sequence includes a flavivirus [6']5' untranslated region (UTR), at least a portion of the 5' coding region for flavivirus core protein, the nucleotide sequence coding for the flavivirus non-structural proteins, and part or all of the 3'-terminal sequence of a flavivirus 3'UTR, required for self-replication of flavivirus genomic material, which vector is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.

60. (once amended) A DNA based replicon vector according to claim 48 wherein the nucleotide sequence is inserted [within the locality]in place of at least a deleted structural gene.